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The yeast integral membrane protein Apq12 potentially links membrane dynamics to assembly of nuclear pore complexes

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Although the structure and function of components of the nuclear pore complex (NPC) have been the focus of many studies, relatively little is known about NPC biogenesis. In this study, we report that Apq12 is required for efficient NPC biogenesis in *Saccharomyces cerevisiae*. Apq12 is an integral membrane protein of the nuclear envelope (NE) and endoplasmic reticulum. Cells lacking Apq12 are cold sensitive for growth, and a subset of their nucleoporins (Nups), those that are primarily components of the cytoplasmic fibrils of the NPC, mislocalize to the cytoplasm. *APQ12* deletion also causes defects in NE morphology. In the absence of Apq12, most NPCs appear to be associated with the inner but not the outer nuclear membrane. Low levels of benzyl alcohol, which increases membrane fluidity, prevented Nup mislocalization and restored the proper localization of Nups that had accumulated in cytoplasmic foci upon a shift to lower temperature. Thus, Apq12p connects nuclear pore biogenesis to the dynamics of the NE.

Introduction

In eukaryotic cells, all nucleocytoplasmic transport occurs through nuclear pore complexes (NPCs), which are large macromolecular assemblies (~44 MD in yeast) that span the nuclear envelope (NE; for review see Tran and Wente, 2006). NPCs show eightfold rotational symmetry in a plane perpendicular to the NE and are constructed using multiple copies of ~30 proteins, which are termed nucleoporins (Nups). Remarkably, more than half of yeast Nups are individually dispensable for growth, although strains lacking some are temperature sensitive for growth and nucleocytoplasmic transport. The nuclear pore itself can be divided roughly into three domains: the nuclear basket, the central core, and the cytoplasmic filaments. The basket and cytoplasmic filaments are composed of Nups that are found solely in those structures, whereas most other Nups are localized symmetrically on both the nuclear and cytoplasmic sides of the plane of the NE. Three integral membrane proteins are components of NPCs and have been implicated in both the organization and proper assembly of NPCs. Although genetic and biochemical analyses have advanced the identification of the Nups as well as their localizations and interactions within the NPC, the mechanism of NPC biogenesis is poorly understood.

Most nucleocytoplasmic transport is mediated by members of the karyopherin family of receptors. These receptors recognize localization signals in their cargoes and move with their cargoes through the central channel of the NPC. mRNA export is not mediated by karyopherins, and the actual complex exported consists of the mRNA in a complex with proteins, forming a messenger RNP complex. The NPC plays a mechanistic role in transport of molecules between the nucleus and the cytoplasm by providing docking sites for these complexes. FG repeat domains are found in approximately one third of yeast Nups and contain FG repeat domains that have multiple copies of GLFG, XFXFG, or XXFG separated by spacers rich in polar amino acids. Structural studies indicate that FG domains are natively unfolded and are able to bind karyopherins and karyopherin–cargo complexes. It is not known how these complexes selectively penetrate the FG repeat milieu of the NPC channel (Denning et al., 2003).

Screening the collection of ~4,500 yeast strains each disrupted for one nonessential gene led to the observation that cells lacking Apq12p have defects in both nuclear 3′ pre-mRNA processing (Baker et al., 2004) and mRNA export (Hieronymus

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Abbreviations used in this paper: BA, benzyl alcohol; $DiOC_6$, 3,3′-dihexyloxacarbocyanine iodide; IF, immunofluorescence; INM, inner nuclear membrane; NE, nuclear envelope; NPC, nuclear pore complex; Nup, nucleoporin; ONM, outer nuclear membrane; SPB, spindle pole body; UPR, unfolded protein response; UPRE, UPR element; WT, wild type.

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et al., 2004). Apq12-GFP localizes to the nuclear periphery and the ER, but it is not a Nup because its distribution is unaffected by mutations that cause NPCs to cluster in one or a few regions of the NE (Baker et al., 2004). More recently, Apq12 was postulated to have a role in cell division, as loss of *APQ12* led to synthetic growth defects when combined with mutations affecting genes coding for spindle pole body (SPB) proteins and other proteins involved in cell division. When Apq12p was not present, anaphase was delayed, and re-replication of DNA before completion of cytokinesis was also observed (Montpetit et al., 2005).

Because of our interest in mRNA biogenesis and export, we examined how the absence of Apq12p affected various aspects of nucleocytoplasmic transport. We report that Apq12p is an integral membrane protein found within the NE and in the ER (Baker et al., 2004). In addition to a partial block in mRNA export, the absence of Apq12p led to cold-sensitive defects in the growth and localization of a subset of Nups, particularly those asymmetrically localized to the cytoplasmic fibrils. In addition, cells lacking Apq12 displayed defects both in NPC biogenesis and in the morphology of the NE. We suggest that these defects are caused by alterations in the dynamics and properties of the NE because the proper localization of Nups in *apq12Δ* cells was restored upon addition to the medium of benzyl alcohol (BA), which is thought to increase the fluidity and flexibility of membranes (Colley and Metcalfe, 1972; Gordon et al., 1980). Thus, it is likely that the reported defects in mRNA export, pre-mRNA processing, and cell division of *apq12Δ* cells are indirect consequences of altered membrane dynamics. Collectively, our results demonstrate the dependence of NPC biogenesis and function on the physical properties of the nuclear membranes.

Results

Apq12p is an integral membrane protein

A previous study demonstrated that Apq12 localizes to the NE/ER and that the protein is not associated exclusively with NPCs (Baker et al., 2004). Hydropathy analyses using the program TMHMM (Krogh et al., 2001) revealed two predicted transmembrane domains (amino acids 40–62 and 69–91). To determine biochemically whether Apq12 is a transmembrane protein, a lysate was prepared from a strain that produces a C-terminally tagged Apq12p-GFP from the genomic *APQ12* locus. Lysates were treated with either Triton X-100, high pH, or buffer alone and subsequently separated into supernatant and pellet fractions by centrifugation. Transmembrane proteins will be found predominantly in the pellet fraction after treatment with high pH or buffer alone. In contrast, high pH treatment causes the release from the membrane of peripherally associated proteins, and they will be found in the supernatant. Immunoblotting of the different fractions with α-GFP antibodies revealed that Apq12-GFP remained in the pellet fraction during high pH treatment and only shifted to the supernatant when lysates were treated with detergent (Fig. 1 A). As controls, Sec23, a peripherally associated ER protein, was found in the supernatant fraction after high pH treatment, whereas the integral membrane ER protein Bos1, like Apq12-GFP, remained in the pellet fraction after high pH treatment. These results prove that Apq12 is an integral membrane protein.

Although *APQ12* is not essential, cells lacking it grow more slowly at 23°C than do wild-type (WT) cells (Fig. 1 B). We compared the growth behavior of *apq12Δ* and WT at 16, 23, 30, and 37°C. *apq12Δ* cells grew as well as WT at both 30 and 37°C

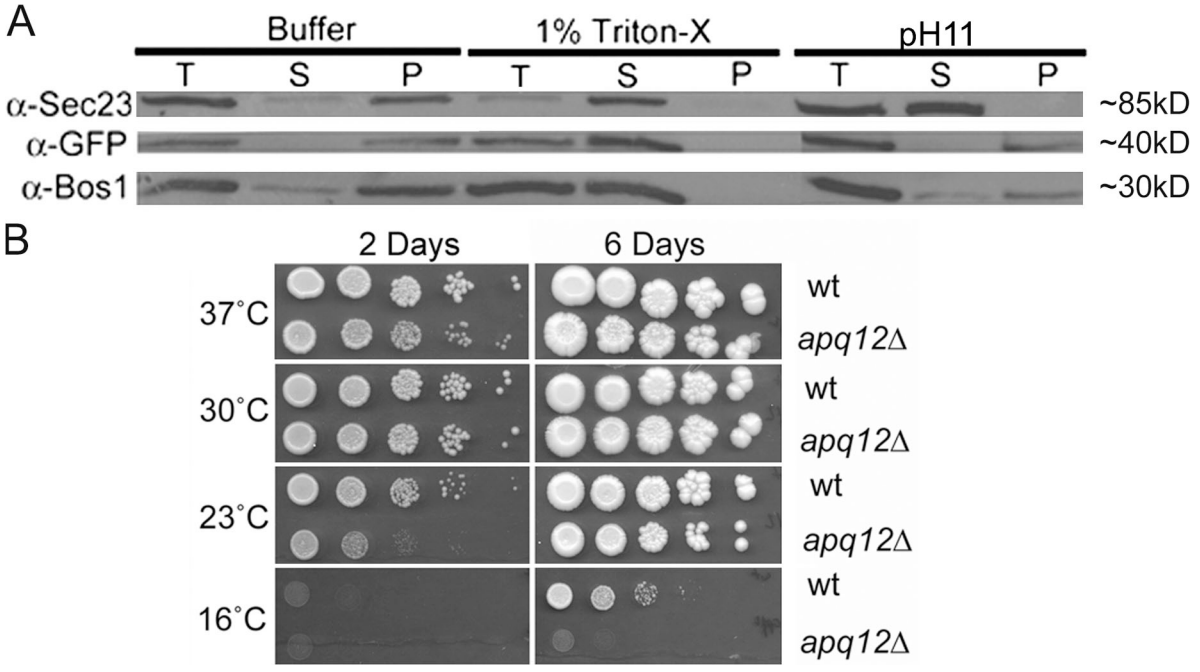

Figure 1. ***APQ12* encodes a transmembrane protein that, when absent, results in cold sensitivity.** (A) Differential centrifugation to determine membrane association. In addition to antibodies against GFP, the blot was also probed using antibodies directed against a control integral membrane protein (Bos1) as well as a peripherally associated membrane protein, Sec23. (B) Growth assays comparing WT and *apq12Δ* cells at various temperatures after 2 and 6 d. T, total; S, soluble; P, pellet.

but were cold sensitive and barely able to grow at 16°C. Notably, the previously described apical cell morphology (Baker et al., 2004) and defect in mRNA export (Baker et al., 2004; Hieronymus et al., 2004) were observed in *apq12Δ* cells grown at 23°C but were not seen in cells maintained at 37°C (see Fig. 3 A).

APQ12 interacts genetically with genes coding for Nups

Because the deletion of *APQ12* led to defects in mRNA export, we investigated whether there were genetic interactions between *apq12Δ* and mutations affecting genes required for nucleocytoplasmic transport. We crossed the *apq12Δ* strain with haploid strains harboring deletions of genes encoding nonessential Nups or ts alleles of genes encoding essential Nups and mRNA export factors, including *rat8-2*, *rat7ΔN/nup159ΔN*, *rss1-37/gle1-37*, *nup120Δ*, and *mex67-5*. Heterozygous diploids were sporulated, tetrads were dissected, and haploid progeny were scored for the presence of both mutations. Growth of double mutant haploid strains was analyzed at temperatures ranging from 23 to 37°C. Of the five mutants listed above, *nup120Δ* had the most severe effect on the growth of *apq12Δ* (Table I). Nup120 is a nonessential structural component of the NPC (Heath et al., 1995). Strong synthetic growth defects were seen with *rat8-2* and *rat7ΔN/nup159ΔN*, but no enhanced growth defect was seen when *apq12Δ* was combined with *mex67-5*. Mex67 is the mRNA export receptor and mediates interactions between the messenger RNP and NPCs during mRNA export (Segref, et al., 1997).

We expanded our genetic analyses to include additional strains in which a gene encoding a nonessential Nup was disrupted. The data are summarized in Table I. Of the others tested, the most severe growth defects were seen when *apq12Δ* was combined with disruptions of *NUP170*, *NUP188*, *NUP85*, *NUP116*, and *POM152*. A strong growth defect was also seen in an *apq12Δ/ndc1-39* strain. Ndc1 is an essential integral membrane protein and is the only protein found in both NPCs and the SPB. Note that little or no defect was seen when *apq12Δ* was combined with either *nup60Δ* or *nup2Δ*. Nup2 and Nup60

Table I. **Synthetic interactions of *apq12Δ***

Relevant genotype	23°C	30°C	34°C	37°C
apq12Δ	++	+++	+++	+++
rat8-2 (dbp5)	++	+++	+	−
apq12Δ/rat8-2	+	+	−	−
mex67-5	++	+++	+	−
apq12Δ/mex67-5	++	+++	+	−
rat7ΔN(nup159ΔN	++	+++	++	−
apq12Δ/rat7ΔN	+	+	+	−
gle1-37(rss1-37)	++	++	−	−
apq12Δ/gle1-37	+	++	−	−
nup42Δ (rip1Δ)	+++	+++	+++	+++
apq12Δ/nup42Δ	+++	+++	+++	+++
nup116Δ	++	++	+	−
apq12Δ/nup116Δ	+	+	+	−
nup120Δ	++	+++	+	−
apq12Δ/nup120Δ	+	+	−	−
nup85Δ	++	+++	+	−
apq12Δ/nup85Δ	+	+	−	−
nup188Δ	+++	+++	+++	+++
apq12Δ/nup188Δ	+	+	+	−
nup170Δ	++	+++	+++	+++
apq12Δ/nup170Δ	+	+	+	−
nup100Δ	+++	+++	+++	+++
apq12Δ/nup100Δ	++	+++	+++	+++
nup59Δ	+++	+++	+++	+++
apq12Δ/nup59Δ	+++	+++	+++	+++
nup2Δ	+++	+++	+++	+++
apq12Δ/nup2Δ	++	++	++	++
nup60Δ	+++	+++	+++	+++
apq12Δ/nup60Δ	++	++	++	++
pom34Δ	+++	+++	+++	+++
apq12Δ/pom34Δ	+	++	++	++
pom152Δ	+++	+++	+++	+++
apq12Δ/pom152Δ	+	++	+	+
ndc1-39	+++	++	+	−
apq12Δ/ndc1-39	+	+	−	−

Synthetic interaction chart for strains that contain the *apq12Δ* disruption in combination with mutations in genes coding for Nups and Nup-associated proteins. +++, WT growth; ++ and +, synthetically sick; −, synthetically lethal.

are components of the nuclear basket of the NPC. Together, these results suggest that Apq12 is important for NPC function or biogenesis.

Loss of Apq12 affects the localization of several Nups

Because of these genetic interactions, we examined the localization of several Nup-GFP fusion proteins in *apq12Δ* cells at 23°C (Fig. 2). We also examined the localization of Nup159/Rat7 and Pom152 by indirect immunofluorescence (IF) using antibodies directed against each protein. Nuclear basket components Nup1 and Nup60 were not mislocalized in *apq12Δ* cells nor were Sac3 or Mlp1, two proteins that associate with nuclear basket Nups (Strambio-de-Castillia et al., 1999; Fischer et al., 2002). Similarly, we observed little or no mislocalization of Nup170, Gle2, Nsp1, or Nup57 in *apq12Δ* cells, all of which are thought to be components of the central structural framework of the NPC. Normal localization was also seen for two integral membrane Nups, Pom152 and Ndc1. Although Nup188, Nup49, and Nic96, which are also core components of the NPC, were not entirely lost from the nuclear periphery, there were subtle differences in their localization in *apq12Δ* cells compared with WT. For example, in cells lacking Apq12, there were studs or bright foci of Nup188- and Nup49-GFP distributed around the nuclear periphery in contrast to their relatively uniform punctate distribution around the nuclear periphery in WT cells.

In contrast to the majority of nuclear basket and core Nups, we observed dramatic defects in the localization of all Nups that are components of the cytoplasmic filaments of the NPC. In the absence of Apq12, Nup42/Rip1, Gle1/Rss1, Nup82, and Nup159/Rat7 mislocalized to foci. Some foci were adjacent to the nuclear periphery, and others were cytoplasmic and appeared to be completely detached from the NE/NPC. We used Western blotting to compare the levels of several Nups in *apq12Δ* and WT cells and saw no differences in those analyzed (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200702120/DC1). In addition to Nup mislocalization, we observed the dramatic mislocalization of Dbp5/Rat8-GFP, an essential mRNA export factor that shuttles between the nucleus and cytoplasm and performs key functions during mRNA export when bound to the cytoplasmic filaments of the NPC (Hodge et al., 1999, Weirich et al., 2004).

To ensure that the mislocalization observed did not result from a synthetic growth defect caused by the presence of a Nup-GFP fusion and the absence of Apq12, we compared the growth of *apq12Δ* cells with several *apq12Δ* strains expressing Nup-GFP fusions. All grew at similar rates (unpublished data). Furthermore, using antibodies directed against the GLFG repeats, we also saw the mislocalization of Nups recognized by this antibody as well as an overall decrease in staining at the nuclear periphery (unpublished data).

Because *apq12Δ* cells grow as well as WT at 37°C (Fig. 1 B), we analyzed at 37°C both mRNA export and the localization of Nups that were mislocalized at 23°C. At this optimal growth temperature, the localization of the cytoplasmic filament Nups (Nup159/Rat7 and Nup82) was normal, and no defect in mRNA export was seen (Fig. 3 A; also see Fig. 6 A).

Both Nup60- and Nup170-GFP were properly localized in *apq12Δ* cells at both 23 (Fig. 2) and 30°C (Fig. 3 B). We shifted WT and *apq12Δ* cells expressing Nup60- or Nup170-GFP from 30 to 16°C and examined their localization after 1 and 2 d. Both Nup170- and Nup60-GFP became abnormally distributed in *apq12Δ* cells while retaining normal distribution in WT cells (Fig. 3 B). Punctate Nup-GFP foci were seen, and the fluorescent signal became more diffuse, with a notable increase in the intranuclear signal. Thus, even Nups whose distribution was normal in slow growing *apq12Δ* cells at 23°C became mislocalized in cells shifted to a more restrictive temperature (16°C). In some *apq12Δ* cells, the nucleus itself became misshapen at 16°C (Fig. 3 B), which is consistent with cold-sensitive defects in the NE. Collectively, the data indicate that Nup localization defects increased in severity and extent as the temperature was reduced.

In the absence of Apq12, the NE is abnormal, and defects in the distribution of NPCs occur

Because Apq12 localizes to the NE and cells lacking Apq12 have NPC defects, we examined the NE by light and electron microscopy. Many ER proteins are present in the outer nuclear membrane (ONM) because the two are continuous. Therefore, we assayed for nuclear membrane deformities by examining the distribution of a GFP fusion to Sec63, a resident ER protein (Prinz et al., 2000), and also by staining live cells with 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$), a fluorescent lipophylic dye that permits easy visualization of ER and nuclear membranes in yeast (Koning et al., 1993). In WT cells maintained at 23°C, both Sec63-GFP (Fig. 4 A) and $DiOC_6$ staining (Fig. 4 B) formed continuous rings surrounding the nucleus. In *apq12Δ* cells, there were several abnormalities, including membranous divisions within the nucleus, extra protrusions of ER membrane not normally seen in WT cells, and studs of fluorescent signal adjacent to the NE.

To gain further insight into the *apq12Δ* defect, we performed electron microscopy to examine NE ultrastructure (Fig. 4, C–H). In WT cells, NPCs appear as electron-dense material extending from the inner nuclear membrane (INM) to the ONM (Fig. 4 C, asterisks). We observed a range of defects in both NPCs and the NE in *apq12Δ* cells. In *apq12Δ* cells grown overnight at 23°C, >90% of NPCs examined contacted only the INM (Fig. 4 E, arrows). Groups of NPCs associated with the NE were seen in some *apq12Δ* cells (Fig. 4 F, arrow), and these probably correspond to the bright fluorescent foci seen in Fig. 3 A and with some Nup-GFPs. Invaginations and/or extensions of the NE (Fig. 4 D) were seen in 30–40% of the nuclei examined. However, because *apq12Δ* cells are able to grow at 23°C, *apq12Δ* cells must have some normal nuclear pores, and these were seen, although rarely (unpublished data). We also observed many cases in which large electron-dense inclusions extended into the lumen of the NE (Fig. 4 G, arrow). Sometimes, these were located entirely within the lumen (Fig. 4 F, arrow).

Because growth at 37°C prevented the Nup82 localization defect seen at 23°C, we also performed electron microscope on cells grown continuously at 37°C. Although NPCs that were

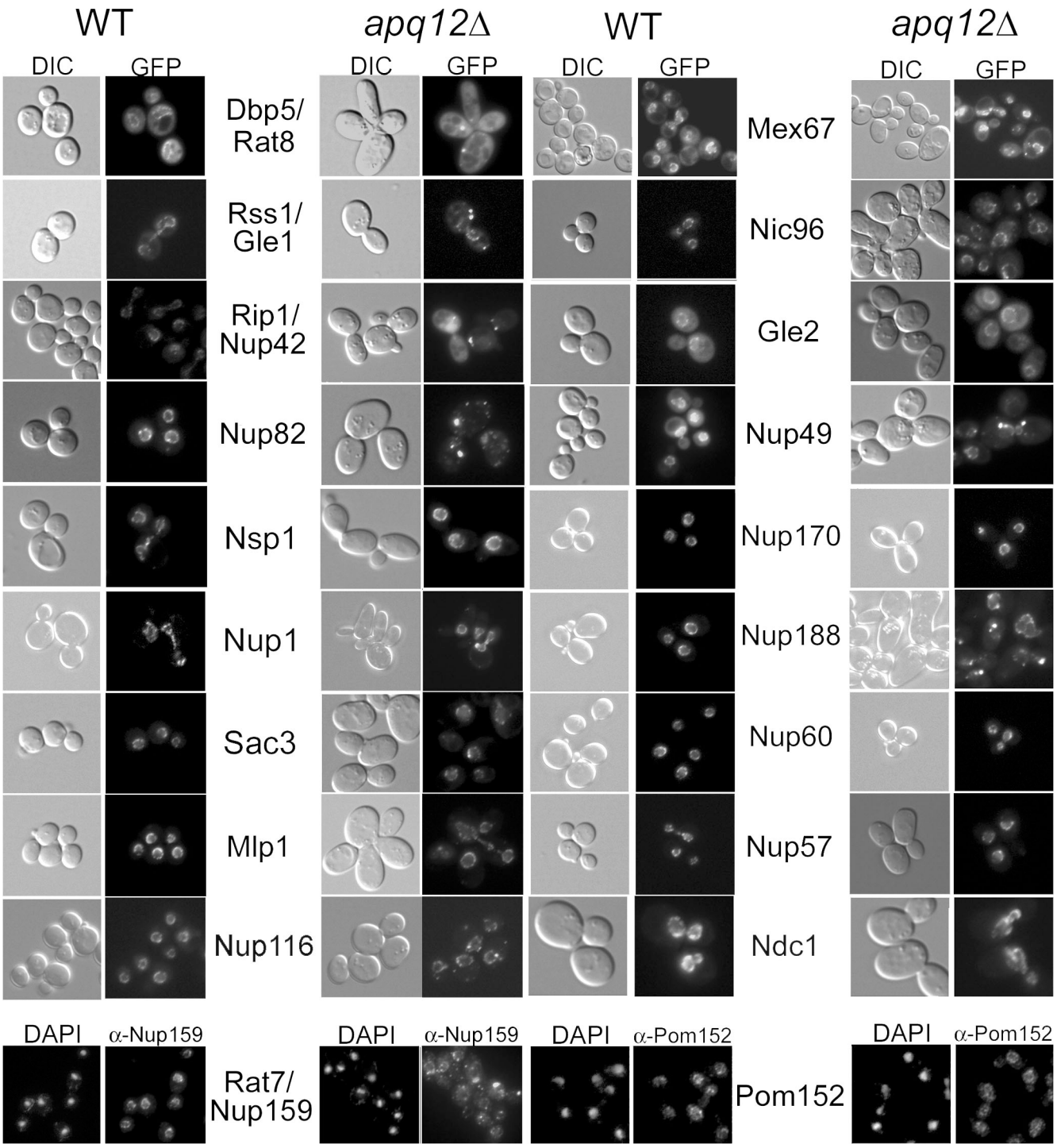

Figure 2. **Loss of *APQ12* causes the severe mislocalization of cytoplasmic filament Nups.** Localizations of various Nups at 23°C in a WT versus *apq12Δ* background. Images for Nup159/Rat7 and Pom152 were from indirect IF experiments using antibodies directed against the respective proteins; all others were viewed via live microscopy using Nup-GFP fusions. DIC, differential interference contrast.

unable to associate with the ONM were still seen (unpublished data), >85% of NPCs appeared normal. Protrusions of the NE that contained electron-dense material (Fig. 4 H, asterisks) were still seen in ~50% of nuclei examined, and only rarely did these contain multiple inclusions, which is in contrast to what was seen at 23°C (Fig. 4 G). More than 200 NPCs were examined in determining these percentages.

Nuclear membrane herniations and membrane seals covering some NPCs are phenotypes associated with the deletion of nonessential *NUP116* (Wente and Blobel, 1993). Because *apq12Δ* cells also showed membrane seals covering some NPCs, we compared the localization of Nup159/Rat7 in *apq12Δ* and *nup116Δ* cells. The data in Fig. 5 show that there was a much more severe defect in the localization of

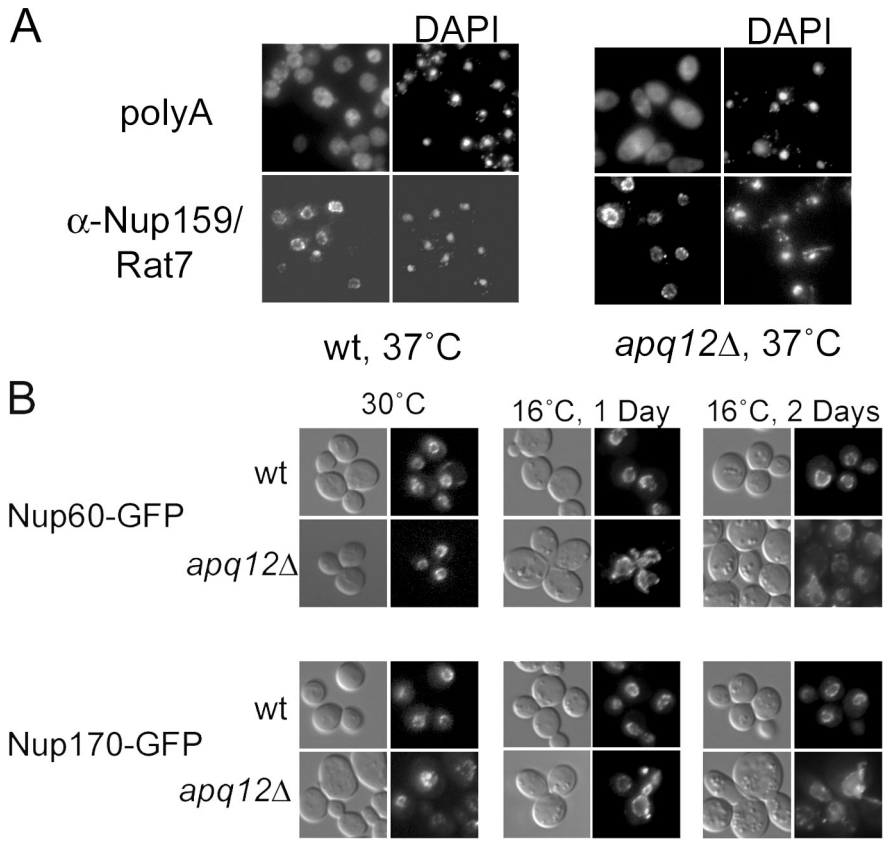

Figure 3. ***apq12Δ* cell growth rates correlate with Nup localization and function.** (A) Images of WT and *apq12*Δ cells processed for either FISH assays or indirect IF using α-Nup159/Rat7 antibodies after overnight growth at 37°C. (B) WT or *apq12Δ* cells expressing either NUP60- or NUP170-GFP from the proper chromosomal locus were grown overnight at 30°C, shifted to 16°C, and viewed after either 1 or 2 d.

Nup159/Rat7 in *apq12Δ* than in *nup116Δ*. We also constructed an *apq12Δ/nup116Δ* double mutant strain, and none of the morphological defects seen in either single mutant were exacerbated by combining the mutations (unpublished data). However, the double mutant strain grew considerably less well than either single mutant at 23 or 30°C and did not grow at 37°C (Table I).

Normal ER function in cells lacking *apq12Δ*

Although there were no readily observed morphological defects in the peripheral ER in *apq12Δ* cells (Fig. 4, A and B), we analyzed *apq12Δ* cells for defects in ER function because Apq12 is present throughout the ER. No defect was observed in the maturation of carboxy-peptidase Y, a recognized cargo of ER protein transport that is modified within the Golgi and is ultimately delivered to the vacuole (Fig. S2 A, available at http://www.jcb.org/cgi/content/full/jcb.200702120/DC1). The accumulation of unfolded proteins in the ER activates the unfolded protein response (UPR; Sidrauski et al., 1998). In yeast strains defective in ER protein trafficking and secretion, cells typically display a constitutively active UPR. The UPR leads to the activation of several genes that encode proteins needed for addressing the increased level of unfolded proteins in the ER. A conserved DNA sequence element (the UPR element [UPRE]) is a feature of promoters activated by the UPR (Mori et al., 1992; Cox et al., 1993). Thus, the expression of GFP from a promoter containing a UPRE has been used as a measure of the extent to which the UPR has been activated in different genetic backgrounds (Pollard et al., 1998; Travers et al., 2000). We saw no induction of a UPRE-GFP reporter in *apq12Δ* cells, although the reporter was activated in the control *erv25Δ* strain (Fig. S2 B).

Apq12 is required for proper NPC biogenesis

Mislocalization of Nups could reflect defects in NPC biogenesis, NPC stability, or both. We took advantage of the cold sensitivity of *apq12Δ* cells to ask whether Apq12 is required for proper NPC biogenesis. First, we determined how the distribution of Nup82-GFP changed over time in cells shifted from 37 to 23°C. We diluted cells grown overnight at 37°C to restore exponential growth and shifted them to 23°C. Mislocalization of Nup82-GFP was detectable but minimal 2 h after the shift (unpublished data) and was complete by 8 h (Fig. 6 A). No mislocalization was seen in *apq12Δ* cells maintained at 37°C.

To distinguish between defects in NPC assembly and stability, we treated cells with the translation inhibitor cycloheximide to prevent the synthesis of new Nups. We reasoned that if mislocalization did not occur in cycloheximide-treated cells, this would indicate that NPCs produced at 37°C were stable at 23°C and that the cytoplasmic foci of GFP-tagged Nups resulted from a defect in NPC assembly. This approach was used earlier by Ryan et al. (2003, 2006) to investigate the genetic requirements for yeast NPC biogenesis. Cells were grown overnight at 37°C, diluted to restore exponential growth, and shifted to 23°C with or without the addition of cycloheximide. As shown in Fig. 6 A, the normal localization of Nup82-GFP was retained in cycloheximide-treated *apq12Δ* cells shifted to 23°C but not in the untreated control. We conclude that *apq12Δ* cells are defective in NPC biogenesis.

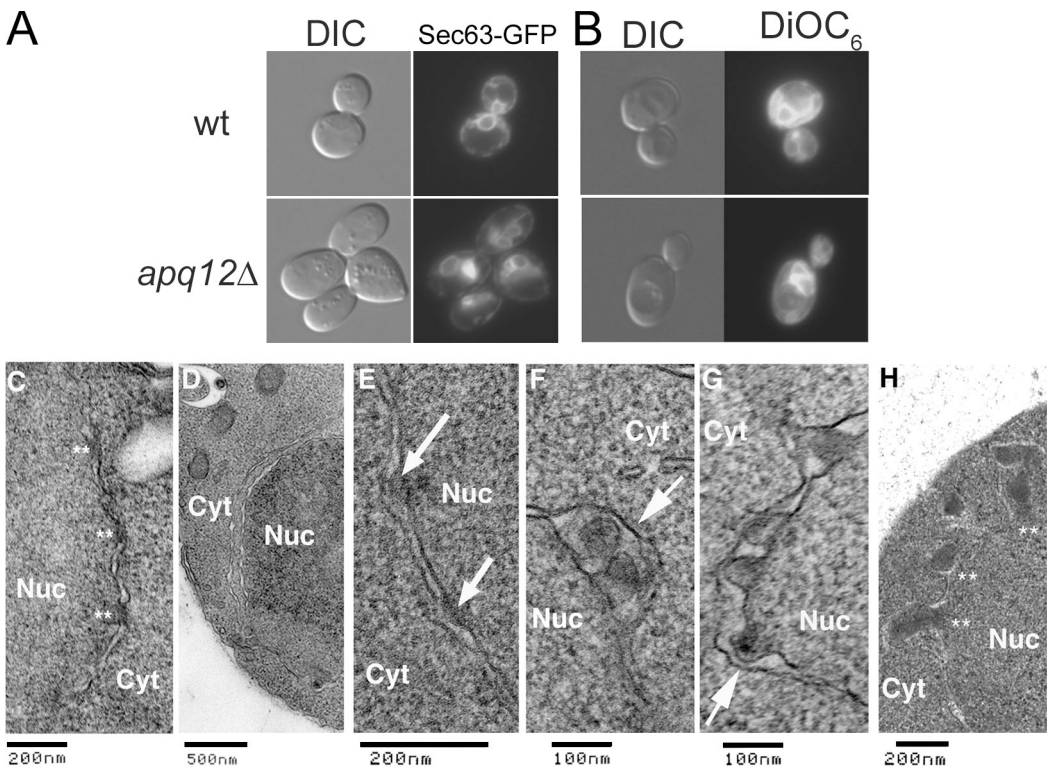

Figure 4. **Loss of *APQ12* causes NE abnormalities.** (A and B) WT and *apq12Δ* cells either harboring a *SEC63-GFP* plasmid (A) or stained with $DiOC_6$ (B) were viewed via live microscopy. (C–G) Electron microscopy images of either WT (C) or *apq12Δ* cells (D–G) at 23°C. (H) *apq12Δ* cells processed for and imaged via electron microscopy after overnight growth at 37°C. Asterisks in C indicate normal NPCs. Arrows in E point to NPCs contacting only the INM. The arrow in F points to an electron-dense inclusion within the lumen of the NE. The arrow in G points to an electron-dense inclusion extending into the lumen of the NE. Asterisks in H indicate protrusions of the NE containing electron-dense material. DIC, differential interference contrast.

As another approach to examine whether Apq12 plays a role in NPC biogenesis, we examined the localization of Nup159/Rat7 (using anti-Nup159/Rat7 antisera) and the various Nups (including Nsp1) recognized by the RL1 monoclonal antibody (Copeland and Synder, 1993) in an *apq12Δ/nup120Δ* double mutant strain (Fig. 6 B). In *nup120Δ* cells, NPCs cluster to one area within the NE (Heath et al., 1995), and both antibodies recognized these clusters (Fig. 6 B; see merge in the *nup120Δ* row). In contrast, in *apq12Δ* cells, RL1 antibody stained the nuclear periphery as well as cytoplasmic foci, presumably as a result of the antibody recognizing some cytoplasmic filament Nups. In the double mutant, very few cells had NPC clusters, and the mislocalization defects seen in *apq12Δ* were enhanced: cytoplasmic foci (stained using RL1) were much brighter in *apq12Δ/nup120Δ* than in *apq12Δ* cells, indicating that additional Nups became mislocalized in double mutant cells, and some accumulated in cytoplasmic foci.

Although both Nup159/Rat7 and Gle1/Rss1 are located asymmetrically on the cytoplasmic side of NPCs, a direct interaction between them has not been detected. However, indirect IF indicated that these two Nups colocalized to cytoplasmic foci in *apq12Δ* cells (unpublished data). Further investigation

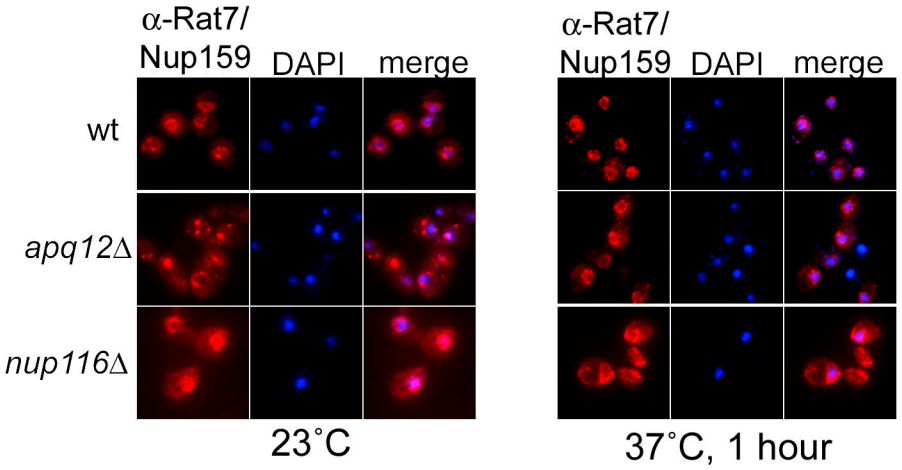

Figure 5. ***nup116Δ* cells display normal localization of Nup159/Rat7.** WT, *apq12Δ*, and *nup116Δ* cells were grown overnight at 23°C, either maintained at 23°C or shifted to 37°C for 1 h, and processed for indirect IF using α-Nup159/Rat7 antibodies.

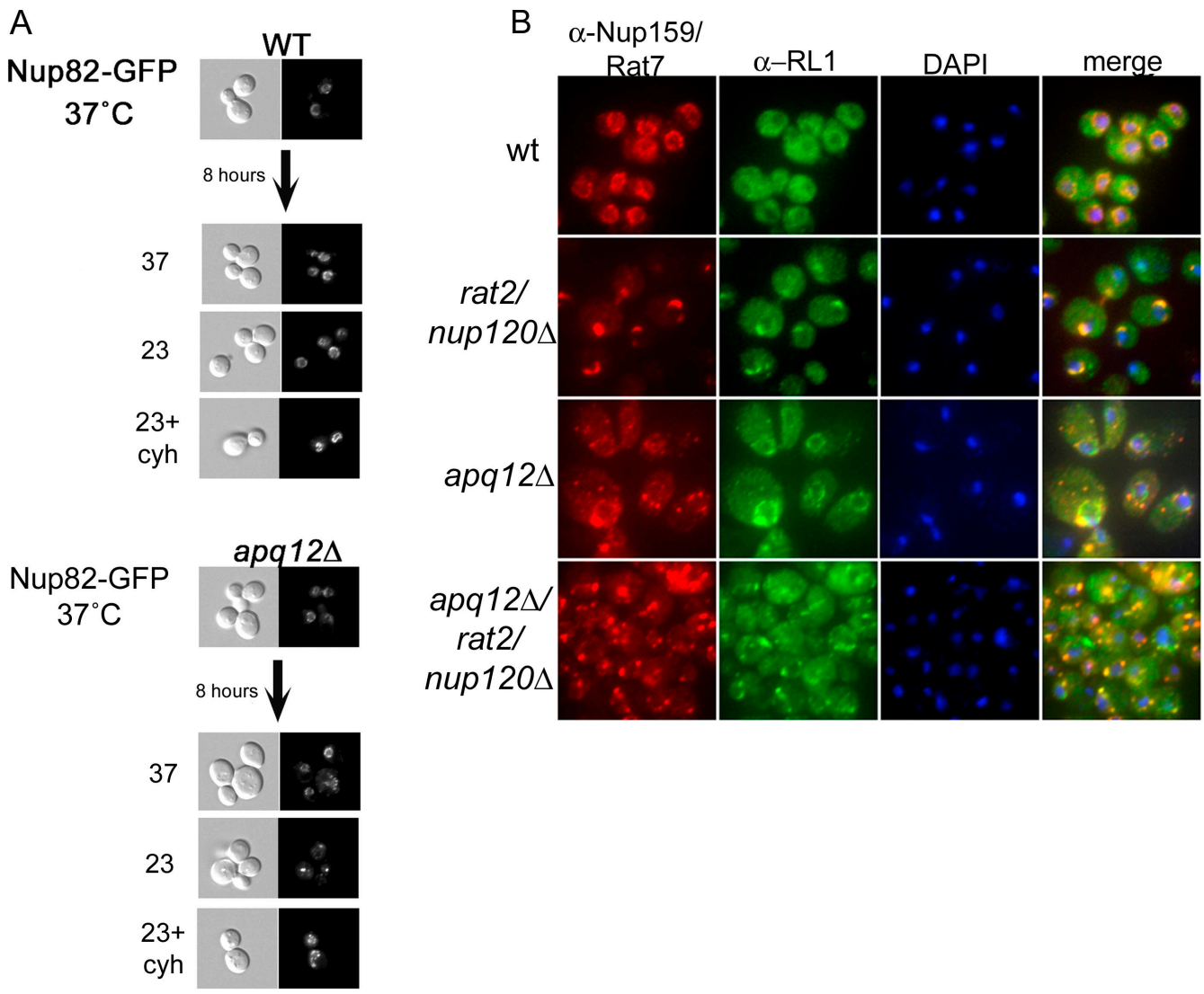

Figure 6. **Apq12 is required for optimal NPC biogenesis.** (A) WT or *apq12*Δ cells expressing Nup82-GFP were grown overnight at 37°C and either maintained at 37°C or shifted to 23°C with or without the translational inhibitor cycloheximide. Cells were then imaged after 8 h. (B) WT and *apq12*Δ cells lacking *NUP120* were grown overnight at 23°C and processed for indirect IF using both α-Nup159/Rat7 and α-RL1 antibodies.

of these foci using indirect IF also demonstrated the colocalization of Nup82-GFP with Nup159/Rat7 and with a fraction of Nup170-GFP (Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200702120/DC1). We suggest that the foci contain Nup subcomplexes that are unable to be assembled into NPCs.

Alteration of membrane dynamics suppresses Nup mislocalization in *apq12Δ* cells

The aforementioned results suggest that the observed mislocalization of some Nups and the defect in NPC biogenesis might result from altered physical properties of the NE. Cold sensitivity is a phenotype sometimes observed with defective membrane proteins (Baba et al., 1990; Lau et al., 2004), and *apq12Δ* cells are cold sensitive for the growth and proper organization of NPCs within the NE. One mechanism cells use to cope with a reduction in growth temperature is to modify the composition of membranes by increasing the abundance of phospholipids that contain shorter and unsaturated acyl chains (Nishida and Murata, 1996). This allows the maintenance of membrane fluidity and flexibility at lower temperatures. Alterations in the protein components of membranes may also contribute to the maintenance of normal membrane properties after temperature shifts. Recently, it was demonstrated that ethanol-induced changes in fluidity of the NE in yeast cells caused defects in both NPC organization and nuclear transport (Izawa et al., 2004). This suggests that the *apq12*Δ-associated phenotypes might be caused by the mutant strain's inability to modulate membrane composition at lower temperatures. To test this hypothesis, we added low levels of BA to the media to increase membrane fluidity (Colley and Metcalfe, 1972; Gordon et al., 1980). *apq12Δ* cells expressing Nup82-GFP were incubated overnight at 37°C and shifted to 23°C for 10 h with or without 0.4% BA. In cells cultured at 23°C with BA, the mislocalization of Nup82-GFP was prevented (Fig. 7 A). This suggests that *apq12Δ* cells are defective in adjusting the composition of

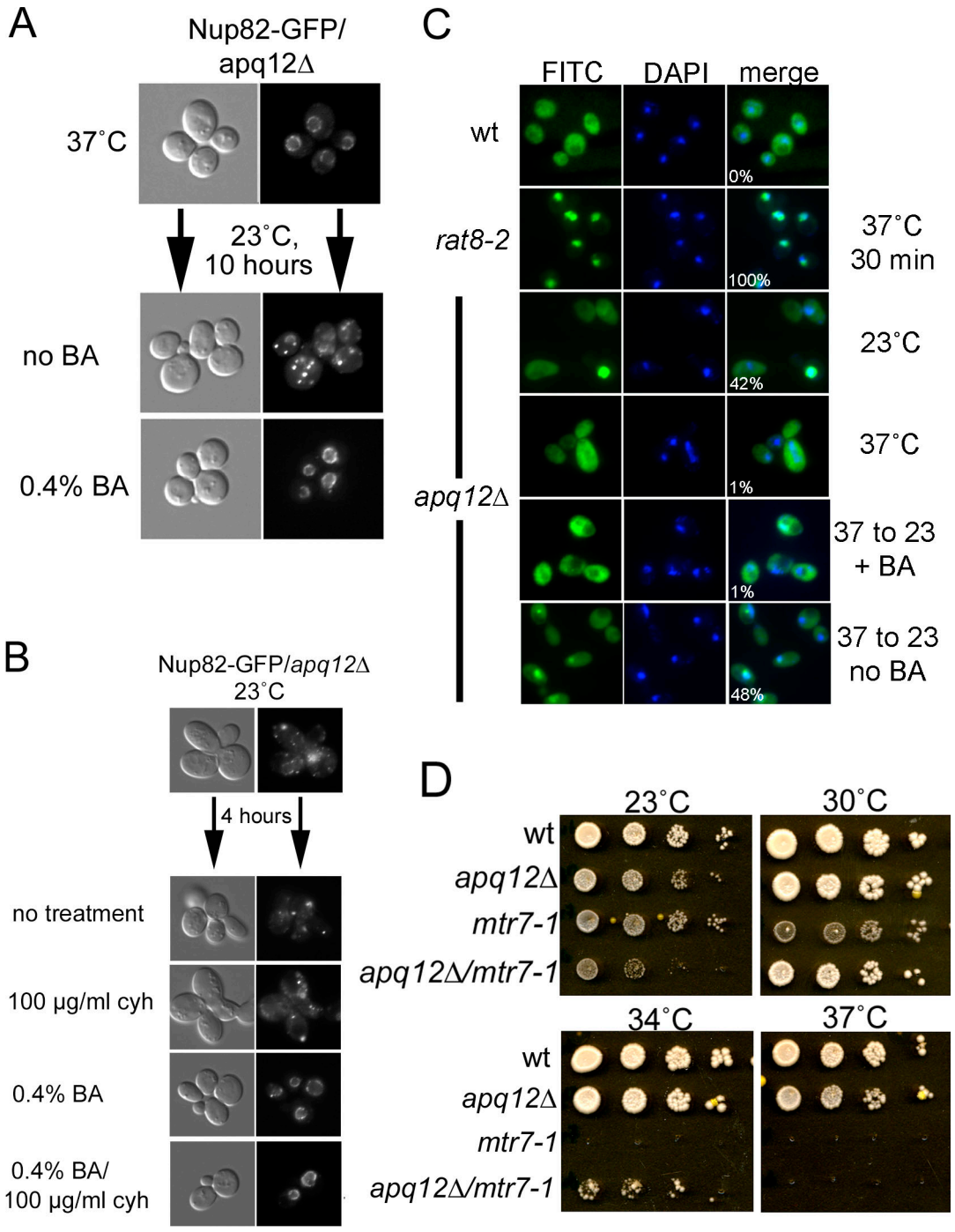

Figure 7. **In *apq12Δ* cells, the membrane fluidizer BA suppresses defects in NPC biogenesis and mRNA export.** (A) *apq12Δ* cells expressing Nup82-GFP were grown overnight at 37°C and shifted to 23°C in the presence (0.4% BA) or absence (no BA) of BA. These cells were then imaged after 10 h at 23°C. (B) *apq12Δ* cells expressing Nup82-GFP were grown overnight at 23°C, treated with either BA, cycloheximide, or both, and imaged after 4 h of treatment. (C) analysis of mRNA export in *apq12Δ* cells treated with BA before the shift to 23°C. (D) Growth assay comparing WT, *apq12Δ*, *mtr7 –1(acc1)*, and the double mutant *apq12Δ/mtr7-1* strains. Plates were incubated at the temperatures shown for 3 d.

the NE so as to maintain proper flexibility or fluidity at lower growth temperatures.

We extended this experiment by adding BA to cells that had been grown overnight at 23°C so that Nup82-GFP was already mislocalized when BA was added (Fig. 7 B). Within 4 h of BA addition, cells had regained the normal localization for Nup82-GFP at the nuclear periphery, suggesting that modifying membrane properties could correct Nup mislocalization. This normal pattern was also restored if cycloheximide was added simultaneously with BA. This indicates that restoration of the normal pattern reflects assembly into NPCs of Nups or Nup complexes already present and mislocalized in cells grown overnight at 23°C. Cycloheximide added without BA had no effect on previously mislocalized Nup82-GFP. We repeated this

assay with an *apq12Δ* strain expressing both a Nup82-GFP fusion and a Sec63-RFP (fusion from their respective chromosomal loci) so that NPC localization and ER morphology could be monitored simultaneously. As shown in Fig. S4 A (available at http://www.jcb.org/cgi/content/full/jcb.200702120/DC1), the morphology of the NE was restored to near normal, but some defects in Sec63-RFP localization were still observed.

We next wanted to determine whether the properly localized NPCs in *apq12Δ* cells treated with BA were functional. To do this, we examined mRNA export in BA-treated *apq12Δ* cells. Only a low percentage of these cells exhibited a defect, suggesting that BA treatment had enhanced the formation of functional NPCs in a*pq12* cells (Fig. 7 C). Likewise, BA corrected the mislocalization of Nup188-GFP in *apq12Δ* cells (Fig. S4 B). Because extended exposure to 0.4% BA had deleterious effects on the growth of WT cells, we were unable to analyze the ability of BA to suppress the cold-sensitive growth defect of apq12*Δ* cells.

Nup mislocalization has been observed in other mutant yeast strains. Both *brr6-1* and *prp20-G282S* cells accumulate Nups in cytoplasmic foci, similar to what was seen with *apq12Δ*. *BRR6* was identified in a screen for cold-sensitive mutants defective in mRNA export and, like *APQ12*, encodes an integral membrane protein of the NE and ER (de Bruyn Kops and Guthrie, 2001). *prp20-G282S* is a novel allele of *PRP20*, encoding the guanine nucleotide exchange factor for Gsp1 (Ran), and, unlike other *prp20* alleles, this mutation is unique in that it causes defects in NPC biogenesis. In contrast to other mutant alleles, *prp20-G282S* does not affect nuclear transport in general (Ryan et al., 2003). The addition of BA was able to partially correct for the mislocalization of Nic96-GFP and Nup170-GFP in the *prp20*-G282-S strain (Fig. S4 C). Little change was seen in the location of Nup159 in *brr6-1* cells shifted to 16°C (Fig. S4 D).

Genetic interactions occur between the *mtr7-1* mutation and *apq12Δ*

The observation that the addition of BA suppresses the NPC biogenesis and mRNA export defects of *apq12Δ* cells suggests that the properties and composition of the NE are affected directly by the absence of Apq12. The *mtr7-1* allele of *ACC1* (encoding acetyl-CoA carboxylase) is known to have defects in the lipid composition of its membranes. Acc1/Mtr7 catalyzes the rate-limiting step in synthesis of fatty acids, and the *mtr7-1* mutant was particularly defective in the synthesis of very long chain fatty acids. We constructed a strain carrying both *apq12Δ* and *mtr7-1* and examined its growth at several temperatures (Fig. 7 D). At 23°C, the double mutant strain grew less well than either single mutant, but at both 30 and 34°C, the double mutant strain showed enhanced growth in comparison with the *mtr7-1* single mutant. At 37°C, both the double mutant and *mtr7-1* cells could not grow. The enhanced defects at lower temperature suggest that double mutant cells are less able to respond to a reduction in temperature and support the hypotheses that both Apq12 and Acc1/Mtr7 affect the composition and, therefore, the properties of the NE. Interestingly, at 23°C, the *apq12Δ/mtr7-1* double mutant had a more severe mRNA export defect than did *apq12Δ* cells (unpublished data).

The cell division defects resulting from the absence of Apq12 are independent of mRNA export defects of *apq12Δ*

During interphase, the nucleus maintains a relatively stable morphology, which is in contrast to the dynamic changes it undergoes in location and structure during cell division. These alterations can be observed easily by staining live cells with $DiOC_6$ during various stages of the cell cycle (Koning et al., 1993). *apq12Δ* cells are known to have cell shape and cell division defects (Montpetit et al., 2005). We wondered whether these phenotypes might be related to and were a consequence of defects in the NE and NPCs.

Nup170 is a structural component of NPCs (Aitchison et al., 1995). Much like Apq12, Nup170 has also been implicated in playing a role in cytokinesis, as the mutation of *NUP170* leads to defects in chromosome segregation and kinetochore integrity (Kerscher et al., 2001). Cells carrying deletions of both *apq12* and *nup170* grew considerably less well at 23°C than either single deletion mutant, and synthetic lethality between *apq12Δ* and *nup170Δ* was seen at 37°C (Fig. 8 A). Because the absence of either Apq12 or Nup170 leads to defects in cell division and nuclear transport/structure, we saw this dramatic synthetic growth defect as an opportunity to determine whether or not there was interdependence between the two defects. We assayed both single mutants and the *apq12Δ/nup170Δ* double mutant for defects in mRNA export and Nup159/Rat7 localization. Surprisingly, double mutant cells were no more defective for mRNA export at 23°C than *apq12Δ* cells (Fig. 8 B), and the mislocalization of Nup159/Rat7 seen in *apq12Δ* cells was also reduced in double mutant cells at 23°C (unpublished data). We next assessed whether or not the deletion of *NUP170* enhanced the cell division defects of *apq12Δ*. Fig. 8 C shows that double mutant cells were morphologically more defective than either of the parental mutants, and acquired a pseudohyphal appearance. $DiOC_6$ staining of double mutant cells showed that the deletion of *NUP170* enhanced the *apq12Δ* nuclear dynamic defects during division, and many cells appeared to have multiple nuclei (unpublished data). Collectively, the data indicate that the deletion of *NUP170* enhanced the nuclear membrane and cell division defects of *apq12*Δ cells, had relatively little effect on the mRNA export defect of *apq12Δ* cells, and partially suppressed the Nup mislocalization defect of *apq12Δ* cells. These results suggest that the *apq12Δ* defects in cell division and NPC function do not depend on each other and are both consequences of NE abnormalities.

Discussion

Because of our long-standing interest in mRNA export, the reported defect in mRNA export in *apq12Δ* cells (Baker et al., 2004; Hieronymus et al., 2004), and the synthetic lethality we observed between *apq12Δ* and *rat8-2*, we decided to investigate the function of Apq12. Apq12 is an integral membrane protein (Fig. 1) present in both the ER and NE (Huh et al., 2003; Baker et al., 2004). *apq12Δ* showed synthetic growth defects when combined with the deletion of any one of several nonessential Nups and with ts alleles affecting several essential Nups (Table I).

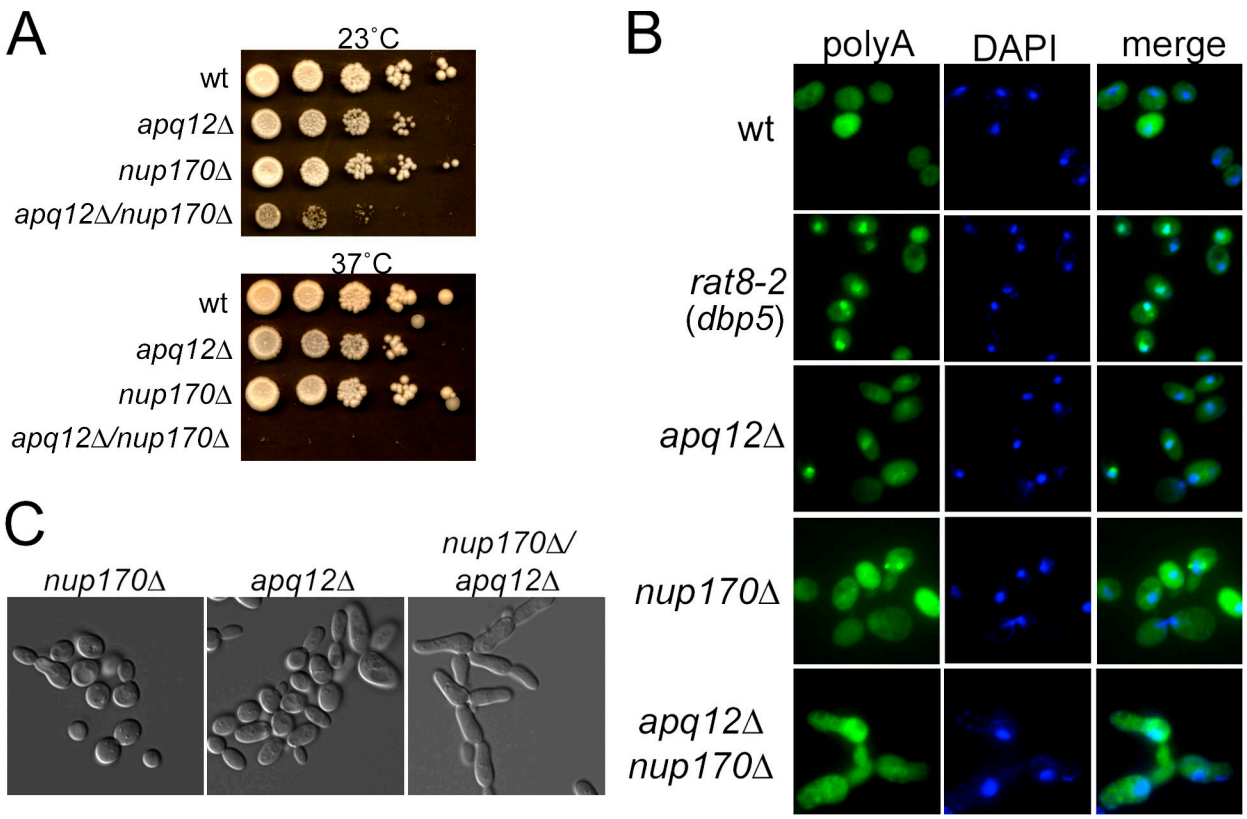

Figure 8. **Defects in NEs/NPCs may also be the cause of *apq12Δ*-associated defects in cell division.** (A) Growth assays comparing the growth of WT, *apq12Δ*, *nup170Δ*, and *apq12Δ/nup170Δ* at 23 and 37°C. (B) FISH of various strains after growth overnight at 23°C. (C) Differential interference contrast images of *nup170Δ*, *apq12Δ*, and *nup170Δ/apq12Δ* cells after growth overnight at 23°C.

The absence of *APQ12* led to defects in NPC biogenesis and the mislocalization of several Nups, including those that comprise the cytoplasmic filaments of the NPC (Nup159/Rat7, Nup82, Gle1/Rss1, and Nup42/Rip1; Fig. 2). Dbp5/Rat8, which binds to these filaments, was also mislocalized in *apq12Δ* cells. Nups that were not NPC associated in *apq12Δ* cells could be detected in foci that contained multiple Nups and Dbp5/Rat8 (Fig. 6 B and not depicted). Strikingly, the Nups in these aggregates retained the ability to be incorporated into NPCs, as the addition of BA restored their localization to NPCs under conditions in which no new Nups could be synthesized (Fig. 7 B).

NPC biogenesis and nuclear membrane dynamics

It is not surprising that some Nups are essential for early steps in NPC biogenesis. Pom152, Pom34, and Ndc1 are transmembrane Nups enriched in NPC-containing subcellular fractions (Rout et al., 2000). Of these, only Ndc1 is essential, and it is also found in SPBs. It is thought that transmembrane Nups initiate NPC construction within the NE and coordinate the early steps of NPC biogenesis, but little is known about how these Nups find one another or the extent to which Nups associate into subcomplexes before their assembly into NPCs. The deletion of *POM152* or mutation of *NDC1* causes genetic defects when combined with the mutation of *NIC96* (Aitchison et al., 1995; Lau et al., 2004), a Nup with a demonstrated role in NPC assembly (Zabel et al., 1996). Similarly, perturbations of NPC structure and function were seen in *pom34Δ/nup188Δ* double mutant cells (Miao et al., 2006). Electron microscopy studies revealed that the pores of *ndc1/pom152* double mutant strains had larger diameters than WT cells, and, as a consequence, NPC transport was perturbed (Madrid et al., 2006). This suggests that in addition to a role in biogenesis, these proteins contribute to the overall structural integrity of the pore.

NPC biogenesis also depends on factors that are not components of NPCs. Recent work suggests that the Ran cycle is required for targeting NPC components such as integral membrane Nups to the NE via a vesicular intermediate (Ryan et al., 2003). Mutations affecting Brr6, another NE protein, result in the altered localization of some Nups (de Bruyn Kops and Guthrie, 2001). Like Apq12, Brr6 was not found in NPC-containing subcellular fractions (Rout et al., 2000) and does not cluster in yeast mutant strains where NPCs cluster. Defects in lipid metabolism have effects on the NE that lead to defects in nuclear transport, presumably by affecting NPCs. An earlier screen for mRNA export mutants identified *mtr7-1*, a temperature-sensitive allele of acetyl coA carboxylase (Acc1/Mtr7; Schneiter et al., 1996). Some of the gross abnormalities of the NE observed in *mtr7-1* cells (Kadowaki et al., 1994; Schneiter et al., 1996) were similar to what we observed in *apq12Δ* cells (Fig. 4). It is likely that the mRNA export defect of *acc1/mtr7* mutants is an indirect consequence of defects in the nuclear membrane that impact NPC biogenesis and function. We hypothesize that this is also the case for *apq12Δ*.

Biological membranes respond to changes in temperature. Membranes contain a diverse mixture of lipids, and phospholipid acyl chains vary in length and in degree of saturation. By an unknown signaling mechanism, cells perceive changes in temperature, and one response is the alteration of membrane lipid composition. A downward shift in growth temperature generally leads to an increase in the proportion of shorter acyl chains and unsaturated acyl chains in membrane phospholipids, resulting in the maintenance of normal fluidity and membrane functions at lower temperatures (Nishida and Murata, 1996). It has been suggested that the cells have a sensor that responds directly to altered fluidity or flexibility by activating the expression of genes needed to modify membrane composition (Vigh et al., 1993).

Cells lacking Apq12 are cold sensitive (Fig. 1 C) and display more severe defects in NE morphology and nuclear transport when incubated at lower temperatures. The finding that BA, a known membrane fluidizer, can prevent the mislocalization of Nups and suppress the mRNA export defect (Fig. 7) suggests that *apq12Δ* cells may be defective in the ability to adjust membrane lipid composition so as to retain the appropriate fluidity and flexibility. This hypothesis is supported by the observed genetic interactions between *apq12Δ* and *mtr7-1* (Fig. 7). It also noteworthy that *apq12Δ* has a strong genetic interaction with *ndc1-39* and *pom152Δ* (Table I), two transmembrane Nups. Although *apq12Δ* has genetic interactions with several nonmembrane Nups, there are many Nups with which it shows little or no genetic interaction.

It is interesting that BA was able to partially suppress the Nup mislocalization defect of *prp20-G282S*. This defect is unique to this particular allele of *PRP20*, which was isolated in a screen for mutants defective in NPC assembly that identified mutants based on Nup mislocalization. Prp20 is not thought to be associated directly with the NE. BA did not lead to any change in the NPC clustering seem in *nup120Δ* cells (unpublished data). This indicates that the absence of Nup120 does not affect the NE in the same way that the absence of Apq12 does even though *nup120Δ* cells have abnormalities of the NE, including protrusions and invaginations. There was also no effect of BA on Nup mislocalization in *brr6-1* cells. Brr6 is an NE protein, and the phenotypes of *brr6-1* cells are similar to those of *apq12Δ* cells.

Defects in nuclear transport, nuclear morphology, and in the NE were also observed in *npl4* mutant strains (DeHoratius, and Silver, 1996). Npl4 along with Cdc48 and Ufd1 form a complex that is involved in the ER-associated proteolysis of some ubiquitinated proteins (Hitchcock et al., 2003). Among the substrates of this complex are the subunits of a heterodimeric transcription factor, Mga2 and Spt23. These proteins are released from a membrane-bound state and are thereby activated by the Npl4–Cdc48–Ufd1 complex (Hitchcock et al., 2001). One of the targets of Mga2/Spt23 is Ole1, which encodes the sole fatty acid desaturase in yeast, and this protein cannot be produced in *npl4* mutant strains. This suggests that the nuclear membrane defects of *npl4* cells arise as a consequence of their inability to activate *OLE1* and that the nuclear transport defects of these cells are an indirect consequence of defects in the nuclear membrane.

Why would a protein that affects membrane dynamics have such a dramatic defect in NPC biogenesis? As shown recently by electron tomography, there are intimate contacts between the NE and NPCs. NPCs have a flexible structure that undergoes conformational and positional changes within the NE during transport (Stoffler et al., 2003; Beck et al., 2004; Melcak et al., 2007). Although the details of NPC biogenesis remain unknown, a critical event is the fusion of the INM and ONM, resulting in the formation of a protein-lined membrane tunnel connecting the nucleus and the cytoplasm. Formation of a membranous tunnel represents a dramatic reorganization of the INM and ONM, and one might expect the formation of NPCs to be quite sensitive to altered biophysical properties of the nuclear membranes.

Nuclear membrane dynamics and cell division

Another study reported that cells lacking Apq12 had defects in the completion of cytokinesis and showed that these defects were independent of *apq12Δ*'*s* mRNA export defect (Montpetit et al., 2005). They identified *apq12Δ* in a screen for mutants that are synthetically lethal with ts alleles affecting SPB proteins. In contrast to what was observed for NPCs, we did not detect the mislocalization of any of the several SPB components we examined (unpublished data).

Moreover, through the study of an *apq12Δ/nup170Δ* double mutant, we were able to separate *apq12Δ*'*s* defects in cell division and mRNA export (Figs. 8 and S1). Nup170 has been linked previously to chromosome segregation (Kerscher et al., 2001). Combining the two deletion mutations exacerbated the defects in cell division and NE morphology, whereas the mRNA export and NPC biogenesis defects shown by *apq12Δ* cells were not increased. One possibility is that when Nup170 is absent, less physical stress is placed upon the nuclear membranes as they undergo fusion. Little is known about the steps of NPC biogenesis, but several Nups may associate with the INM and ONM before fusion. In the absence of Nup170, perhaps less bending of the membranes is required in forming the NPC tunnel, and this would account for reduced Nup mislocalization in *apq12Δ/nup170Δ* cells. If this were the case, one would expect that mRNA export would be less defective in the double mutant than in *apq12Δ* cells, as we observed. Entirely different demands are likely placed upon the NE during cell division than during NPC biogenesis. The presence of a less flexible NE could readily enhance the cell division defect in *apq12Δ/nup170Δ* cells as compared with the defect of *nup170Δ* cells.

We speculate that the cell cycle defects of *apq12Δ* are also a consequence of altered nuclear membrane properties. Yeast cells undergo a closed mitosis, and NE breakdown does not occur. Instead, after chromosome duplication, cells undergo karyokinesis before cytokinesis. This involves movement of the nucleus to the bud neck and elongation of the nucleus into the bud followed by severe constriction of the NE as karyokinesis proceeds and the mother and daughter cells begin to become separated by cytokinesis. This dramatic remodeling of the NE is likely to be impacted by mutations that affect NE flexibility.

Interestingly, mutations in *ACC1/MTR7* also cause defects in mitosis, but *acc1*/mtr7mutants are sensitive to elevated as well as reduced temperatures. At nonpermissive temperature, *acc1/mtr7* cells arrest at the G2/M boundary with a single nucleus and a short spindle, and this could reflect an inability of the NE to undergo the changes in nuclear shape required for karyokinesis (Al-Feel et al., 2003).

Collectively, these data support the hypothesis that the biophysical properties of the nuclear membrane are affected by the loss of Apq12, and this leads to defects in NPC biogenesis. The reported defects in nuclear transport and RNA metabolism are consequences of the defects in NPC biogenesis. Although a detailed mechanistic role for Apq12 has not been defined, there are several possibilities for how it may be acting. Because Apq12 is present in the NE, it could contribute directly to the biophysical properties of the nuclear membranes as a structural component. Alternatively, Apq12 could be required to sense the physical properties of the NE so that the cell is signaled to modify its membranes after temperature changes. Also, Apq12 might function downstream of a sensor to recruit enzymes involved in lipid biosynthesis or modification. These include biosynthetic enzymes such as Acc1/Mtr7 and the desaturase Ole1. Defining the precise role played by Apq12 will be the focus of future studies.

Materials and methods

Yeast strains and plasmids

Yeast strains and plasmids used for these studies are listed in Table S1 (available at http://www.jcb.org/cgi/content/full/jcb.200702120/DC1). All strains were grown and media was prepared using standard methods (Burke et al., 2000). For growth assays, strains were grown overnight and diluted back to OD_{600} = 0.3. Strains were then serially diluted 1:10, and 3 µl were plated of each dilution. Plasmid pCSNup49-GFP-1 was created by the digestion of GFP-NUP49 plasmid (gift of V. Doye, Institut Curie, Paris, France) with SacI and BamHI and ligation into YIplac211. The plasmid was then linearized for genomic integration with SwaI.

Microscopy

All live cell fluorescent microscopy was performed using cells grown and mounted in synthetic complete plus dextrose media (Burke et al., 2000). Images were acquired using a microscope (TE2000-E; Nikon) fitted with a 100× NA 1.4 plan Apochromat oil objective (Nikon), CCD camera (Orca-ER; Hamamatsu), and Phylum Live software version 3.5.1 (Improvision). GFP and RFP were visualized by using an X-cite 120-UV lamp and Chroma filter sets. Images were processed using Photoshop 7.0 (Adobe). $DiOC_6$ staining was performed as previously described (Koning et al., 1993). In brief, cells in midlog phase were stained with 1 µg/ml $DiOC_6$ (Invitrogen) using a 0.1-mg/ml ethanol stock. Indirect IF using α-Nup159/Rat7, α-Gle1/Rss1, or α-RL1 and FISH were performed as described previously (Gorsch et al., 1995; Cole et al., 2002). α-Nup159/Rat7 antibody was used at a 1:3,000 dilution, and α-RL1 (Affinity BioReagents, Inc.) was used at a 1:500 dilution. Indirect IF using α-Pom152 antibodies (Strambio-de-Castillia et al., 1995) was performed as described previously (Wente et al., 1992) using a 1:2 antibody dilution.

Membrane association assay

100 ml Apq12-GFP cells were grown to an OD_{600} = 0.6 and were used for semiintact cell preparation. In brief, cells were washed once in 100 mM Tris-HCl, pH 9.4, and 5 mM DTT and resuspended in 10 mL lyticase buffer (0.7 M sorbitol, 0.5% dextrose, 10 mM Tris-HCl, pH 7.5, and 1 mM DTT). 250 µl lyticase was added, and cells were gently rotated for 15 min, at which time the reaction was stopped by adding 90 mL lyticase buffer and spinning down cells. The pellet was resuspended in 1.8 ml lysis buffer (0.4 M sorbitol, 20 mM Hepes, 150 mM KOAc, and 2 mM MgOAc). 35 µl of the semiintact cells were treated with 115 µl of buffer A (20 mM Hepes, pH 7.0, 150 mM KOAc, and 2 mM EDTA), buffer A plus 1% Triton X-100, or 0.1 M sodium carbonate, pH 11.0, for 5 min and spun at 60 K for 12 min. The supernatant was then removed, the pellet was resuspended, and the fractions were analyzed via Western blotting.

Electron microscopy

Electron microscopy was performed as previously described (Goldstein et al., 1996) with some modifications. In brief, the cells were grown to an OD_{600} of 0.5–1.0 in YPD media, pelleted, and resuspended in 0.1 M cacodylate buffer, pH 6.8. Primary fixation was performed with 3% glutaraldehyde and 0.1% tannic acid in 0.1 M cacodylate buffer, pH 6.8, at room temperature for 1 h and then overnight at 4°C. Cells were washed twice with 0.1 M cacodylate buffer, pH 6.8, and twice with 0.1 M phosphate buffer, pH 7.5, and treated with zymolyase (10 mg/ml 100T) to produce spheroplasts. After washing with phosphate buffer and cacodylate buffer, pH 6.8, the cells were retreated with 3% glutaraldehyde and 0.1% tannic acid in 0.1 M cacodylate buffer, pH 6.8, for 1 h at room temperature, washed three times with 0.1 M cacodylate buffer, pH 6.8, and embedded in low melting temperature agarose (SeaPrep; FMC Corp.). Postfixation was performed with 2% osmium tetroxide in 0.1 M cacodylate buffer, pH 6.8, for 1 h on ice. Subsequently, the cells were washed in cacodylate buffer, pH 6.8, and deionized water, en bloc stained with 0.5% uranyl acetate overnight, dehydrated with ethanol, and embedded in Spurr's resin (medium grade). Thin sections were cut on an ultramicrotome (MT5000; Sorvall) with a section thickness of 100 nm. Sections were poststained with uranyl acetate and Venable and Coggleshell's lead citrate and examined on a transmission electron microscope (JEM 1010; JEOL) at 100 kV.

Online supplemental material

Fig. S1 shows growth assays for some of the double mutants whose growth behavior is summarized in Table I and a comparison of the protein levels of various Nup-GFP fusion proteins in WT and *apq12Δ* cells. Fig. S2 documents that *apq12Δ* cells do not have an ER-trafficking defect, as analyzed by comparing both the maturation of carboxypeptidase Y and the induction of the UPR in WT and *apq12Δ* cells. Fig. S3 contains fluorescence micrographs that document the degree of colocalization of multiple Nups that mislocalize to cytoplasmic foci in the absence of Apq12. Fig. S4 shows how BA affects the distribution of Sec63-RFP and Nup82-GFP in *apq12Δ* cells, Nup188-GFP in *apq12Δ* cells, Nup170-GFP and Nic96-GFP in *prp20G280S* cells, and Nup159/Rat7 in *brr6-1* cells. Table S1 lists the strains and plasmids used in these studies. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200702120/DC1.

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